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Solid state conformation and solution structure of a model dehydro-Phe containing peptide N-Ac-dehydro-Phe-L-Val-OCH_3 in various solvents

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Abstract : The conformation of a model peptide N-Ac-dehydro-Phe-L-Val-OCH_3 has been determined in several solvents with varying polarities and has been found to be similar. The peptide was synthesized by the usual workup procedure. The peptide was crystallized in various solvents such as, benzene, chloroform, ethylacetate, acetonitrile, methanol and dimethylsulfoxide. The conformations of the peptide in these solvents have been found to be similar. These correspond to alternating right-handed and left-handed zigzag conformations with Φ, Ψ values in the vicinity of $\pm 50°, \pm 50°$. The Φ, Ψ values of dehydro-Phe remain particularly unaltered in all the solvents. The NMR results in chloroform ($CDCl_3$) and dimethylsulfoxide (DMSO) indicate that the solution conformations of the peptides are essentially similar in both solvents and the Φ, Ψ values correspond to those observed in solid state. The packings of the molecules have been found to be identical in all the solvents.

Keywords : α,β-dehydro-peptide conformation, dehydro-Phe peptides, X-ray structure, design

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1. Introduction

Recent studies have shown that the dehydro-residues can act as the nucleating centres in the resulting conformations of dehydro-residue containing peptides and have become a useful tool in the design of peptides [1]. The planar dehydro-residues introduce strong constraints on the backbone and restrict the torsion angles at the dehydro-residue to only three sets of Φ, Ψ values : $-60°, 120°; 80°, 0°$ and $-50°, -40°$ or their enantiomeric values. Furthermore,

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Supplementary Material :
The listing of final anisotropic thermal parameters of non-hydrogen atoms, positional parameters and isotropic thermal parameters of hydrogen atoms and tables of structure factors can be obtained from the authors on request.

these values are site specific as a dehydro-residue at $(i + 2)$ position adopts values of Φ, Ψ centred around 80°, 0° while at $(i + 1)$ position its torsional angles are –60°, 120°. The dehydro-residues also impose restrictions on the free rotations of its neighbouring saturated residues which can adopt only limited compatible conformations. Therefore, the dehydro-residues can be substituted selectively to produce various desired structures of peptides.

The dehydro-residue containing peptides have been studied systematically using X-ray diffraction method in crystalline state [1–17] and by NMR techniques in solution phase [17–26]. The consistent results of X-ray diffraction studies have led to the deduction of important conclusions which have far reaching consequences on peptide design [1,16].

On the other hand some earlier studies using nuclear magnetic resonance (NMR) techniques have indicated the existence of different conformations in chloroform ($CDCl_3$) and dimethylsulfoxide (DMSO), thus suggesting the possibility of multiple conformations of the dehydro-residue containing peptides in solution [18–21]. However, recent reports using NMR provide the evidence of the presence of a dominant folded structure in DMSO as observed for β-turn forming sequences in $CDCl_3$ [13,17,22–26]. Therefore, a clear picture is yet to emerge about the conformations of dehydro-residue containing peptides in various solvents using NMR data. To resolve this problem, we have crystallized a dehydro-Phe containing peptide N-Ac-dehydro-Phe-L-Val-OCH_3 in various solvents of greatly different dielectric constants such as : benzene (C_6H_6), chloroform ($CHCl_3$), ethylaccetate ($CH_3COOC_2H_5$), methanol (CH_3OH), acetonitrile (CH_3CN) and dimethylsulfoxide (DMSO) ($(CH_3)_2SO$). It is assumed that the most stable conformation in a particular solvent has high probability to form crystals. The crystal structure and molecular conformation of a peptide N-Ac-dehydro-Phe-L-Val-OH in acetonitrile has been reported earlier [3]. We report here the crystal and molecular structures of a peptide N-Ac-dehydro-Phe-L-Val-OCH_3 in various solvents along with its solution conformations in DMSO and $CDCl_3$.

2. Experimental procedures

The peptide N-Ac-dehydro-Phe-L-Val-OCH_3 was synthesized by usual work up procedure [3]. The peptide samples were crystallized in chloroform ($CHCl_3$), ethylacetate ($CH_3COOC_2H_5$), methanol (CH_3OH), acetonitrile (CH_3CN) and dimethylsulfoxide (DMSO) ($(CH_3)_2SO$) at 25°C, and in benzene (C_6H_6) at 4°C.

The X-ray intensity data for crystals grown from various solvents were collected on a CAD4 diffractometer using graphite monochromated CuK_α radiation with $\lambda = 1.5418$ Å. The Lorentz and polarization corrections were applied. The structures were determined by direct methods using the program SHELXS86 [27], and difference Fourier calculations. The coordinates of non-hydrogen atoms and their thermal parameters for all the structures except that in DMSO were refined anisotropically using a full matrix structure factor least-squares procedure on $|F|$ values [28]. The positions of carbon atoms of the OCH_3 group could not be obtained in the structures from benzene and acetonitrile solvents due to poor electron density for OCH_3. The quality of data on crystals from DMSO was poor as only 539 reflections

($I \geq 3\sigma$) were observed because the crystals were very small. Therefore, the structure in DMSO was refined only isotropically. The z-coordinate of O_1' was kept fixed during the course of refinement in all the structures. The hydrogen atoms in all the structures except in DMSO were fixed geometrically and their positions were compared with the difference Fourier maps. They were assigned the final isotropic thermal parameters of the atoms to which they were bonded. These were included in the final cycles of refinement as fixed parameters. The atomic scattering factors used in these calculations were those of Cromer and Mann [29] for non-hydrogen atoms and of Stewart *et al* [30] for hydrogen atoms. The brief summary about crystal data, the intensity data collection and refinement are listed in Table 1. The atomic coordinates of non-hydrogen atoms in all these structures are given in Table 2.

Table 1. Crystal data and other experimental details for model peptide in different solvents.

	C_6H_6	$CHCl_3$	$CH_3COOC_2H_5$	CH_3OH	CH_3CN	$(CH_3)_2SO$
Dielectric constant	2.30	4.80	6 03	32.0	36 02	46 60
Chemical formula	$C_{16}H_{20}N_2O_4$	$C_{17}H_{22}N_2O_4$	$C_{17}H_{22}N_2O_4$	$C_{17}H_{22}N_2O_4$	$C_{16}H_{20}N_2O_4$	$C_{17}H_{22}N_2O_4$
Formula weight	304.35	318.37	318 37	318.37	304 35	318 37
Crystal system	Hexagonal	Hexagonal	Hexagonal	Hexagonal	Hexagonal	Hexagonal
Space group	$P6_5$	$P6_5$	$P6_5$	$P6_5$	$P6_5$	$P6_5$
a, Å	11.864(2)	11.880(2)	11.865(2)	11.885(1)	11 875(2)	11.889(2)
b, Å	11.864(2)	11.880(2)	11 865(2)	11.885(1)	11.875(2)	11 889(2)
c, Å	21.919(8)	21 944(8)	21.899(8)	21 934(2)	21 856(8)	21 850(8)
d_m, gcm^{-3}	1 156(5)	1 195(5)	1.196(5)	1 196(5)	1.151(5)	1 192(5)
d_c, gcm^{-3}	1.135(3)	1.183(3)	1.188(2)	1 182(2)	1 136(3)	1.186(3)
Crystal size in mm^3	0 9×0 08 ×0.06	0.5×0.04 ×0.02	0.8×0.06 ×0 05	1 0×0.07 ×0 05	1.0×0 08 ×0.06	0.3×0 02 ×0 01
F(000)	972	1020	1020	1020	972	1020
Mode of data collection	ω-2θ	ω-2θ	ω 2θ	ω-2θ	ω 2θ	ω 2θ
Number of observed reflections (i≥3σ)	1045	1069	1603	1294	1922	539
Final R	0.077 anisotropic	0.068 anisotropic	0.076 anisotropic	0.077 anisotropic	0 074 anisotropic	0 14 anisotropic

The ^{1}H NMR spectra were obtained on Bruker AMX-400 machine. The phase sensitive 2D-NMR experiments were used for assignments and for conformation analysis. 2D spectra were recorded for 512 t_1 values with 1024 complex points for each free induction decay. 32 scans per t_1 points were taken. Both +ve and –ve peaks were plotted without discrimination in all COSY and rotating frame NOESY (ROESY) spectra [31]. For ROESY experiments the mixing time used was 300 ms. The lyophilised peptides were used to prepare samples of 5 mM concentration for all experiments.

Table 2A. Final atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for non-hydrogen atoms in benzene ($\varepsilon_r = 2.30$).

Atom	x	y	z	U_{eq}^*
C_1	13890 (10)	10467 (11)	1228 (11)	66 (7)
C_2	12497 (8)	9719 (8)	1399 (11)	57 (5)
O_1	12112 (6)	8945 (6)	183 (10)	71 (4)
N_1	11667 (6)	9902 (6)	1050 (10)	48 (3)
C_1^{α}	10321 (8)	9095 (7)	1153 (10)	51 (4)
C_1^{β}	9502 (8)	9503 (7)	1261 (11)	41 (4)
C_1^{γ}	9653 (8)	10874 (8)	1349 (11)	69 (4)
$C_1^{\delta 1}$	8539 (12)	10834 (10)	1501 (11)	82 (6)
$C_1^{\varepsilon 1}$	8551 (14)	11968 (15)	1589 (12)	137 (10)
C_1^{ζ}	9804 (16)	13123 (12)	1616 (11)	117 (8)
$C_1^{\varepsilon 2}$	10882 (14)	13118 (11)	1530 (12)	91 (8)
$C_1^{\delta 2}$	10811 (11)	11923 (10)	1368 (12)	74 (6)
C_1'	9824 (9)	7703 (8)	1068 (10)	56 (4)
$O_1'^{b}$	8873 (6)	6820 (5)	1379	57 (3)
$N_{2_{\alpha}}$	10386 (7)	7326 (6)	626 (10)	56 (4)
C_2^{α}	9938 (9)	5960 (8)	522 (11)	55 (5)
C_2^{β}	10790 (10)	5816 (9)	44 (11)	80 (5)
$C_2^{\gamma 1}$	12138 (12)	6327 (11)	306 (11)	103 (8)
$C_2^{\gamma 2}$	10210 (12)	4440 (11)	-177 (12)	101 (8)
C_2'	8548 (10)	5384 (10)	273 (11)	71 (6)
O_2'	8210 (9)	5926 (9)	-102 (11)	141 (6)
O_2	7784 (8)	4177 (7)	515 (10)	71 (5)

$$U_{eq}^* = 1/3\left[U_{11}(a\,a^*)^2 + U_{22}(b\,b^*)^2 + U_{33}(c\,c^*)^2 + 2U_{12}\,a\,b\,a^*\,b^*\cos\gamma + 2U_{23}\,b\,c\,c^*\,b^*\cos\alpha + 2U_{13}\,a\,c\,a^*\,c^*\cos\beta\right]$$

[b]The z coordinate of O_1' was kept fixed during refinement.

Table 2B. Final atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for non-hydrogen atoms in chloroform ($\varepsilon_r = 4.80$).

Atom				U_{eq}^*
C_1	486 (13)	6559 (15)	1334 (8)	128 (11)
C_2	-261 (12)	7217 (11)	1169 (6)	64 (7)
O_1	-1018 (9)	6871 (8)	749 (4)	100 (6)

Table 2B *(Cont'd.)*

N_1	–64 (9)	8234 (8)	1517 (5)	56 (6)
C_1^{α}	–905 (10)	8781 (10)	1458 (5)	50 (6)
C_1^{β}	–480 (12)	10104 (12)	1320 (5)	71 (7)
C_1^{γ}	781 (11)	11134 (12)	1219 (6)	66 (7)
$C_1^{\delta 1}$	3094 (15)	12208 (16)	1055 (8)	89 (11)
$C_1^{\varepsilon 1}$	1953 (13)	11153 (11)	1203 (7)	39 (7)
C_1^{ζ}	3168 (14)	13359 (14)	950 (7)	66 (9)
$C_1^{\varepsilon 2}$	2041 (18)	13433 (13)	966 (10)	105 (13)
$C_1^{\delta 2}$	837 (13)	12318 (12)	1112 (6)	65 (8)
C_1'	–235 (11)	7861 (11)	1527 (6)	67 (8)
$O_1'^{b}$	–3147 (7)	7973 (8)	1215	68 (5)
N_2	–267 (8)	693 (8)	1948 (5)	46 (6)
C_2^{α}	–4021 (11)	6062 (12)	2089 (5)	65 (7)
C_2^{β}	–418 (15)	5021 (13)	2551 (6)	105 (9)
$C_2^{\gamma 1}$	3673 (14)	42 (13)	2288 (8)	71 (10)
$C_2^{\gamma 2}$	–5601 (13)	423 (13)	276 (6)	67 (9)
C_2'	–4625 (14)	6883 (15)	2324 (7)	73 (10)
O_2'	–4108 (10)	7732 (10)	2684 (6)	122 (8)
O_2	–5806 (8)	6428 (9)	2110 (5)	97 (6)
C_3	–6417 (23)	7108 (27)	2356 (12)	288 (29)

$$U_{eq}^{a} = 1/3\left[U_{11}(a.a^*)^2 + U_{22}(b\,b^*)^2 + U_{33}(c\,c^*)^2 + 2U_{12}\,a\,b\,a^*\,b^*\cos\gamma + 2U_{23}\,b\,c\,c^*\,b^*\cos\alpha + 2U_{13}\,a\,c\,a^*\,c^*\cos\beta\right]$$

[b]The z coordinate of O_1' was kept fixed during refinement.

Table 2C. Final atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for non-hydrogen atoms in ethylacetate ($\varepsilon_r = 6.03$).

Atom	x	y	z	U_{eq}^{a}
C_1	3402 (8)	–487 (8)	887 (5)	85 (6)
C_2	2786 (7)	311 (7)	724 (4)	70 (5)
O_1	3159 (5)	1048 (5)	270 (3)	74 (3)
N_1	1764 (5)	83 (5)	1067 (3)	46 (3)
C_1^{α}	1217 (6)	899 (6)	971 (3)	58 (3)
C_1^{β}	–46 (6)	479 (6)	859 (4)	49 (4)
C_1^{γ}	–1123 (6)	–782 (6)	744 (3)	55 (4)

Table 2C *(Cont'd.)*

$C_1^{\delta 1}$	-1112 (7)	-1932 (8)	750 (4)	57 (5)
$C_1^{\varepsilon 1}$	-2211 (12)	-3096 (9)	582 (5)	113 (7)
C_1^{ζ}	-3358 (9)	-3130 (10)	496 (5)	69 (6)
$C_1^{\varepsilon 2}$	-3417 (8)	-2034 (12)	498 (5)	96 (7)
$C_1^{\delta 2}$	-2331 (8)	-866 (9)	628 (5)	82 (6)
C_1'	2155 (6)	2353 (6)	1038 (3)	54 (3)
$O_1'^{b}$	2066 (5)	3173 (4)	752	67 (3)
N_2	3069 (5)	2678 (5)	1475 (3)	52 (3)
C_2^{α}	3955 (6)	4036 (6)	1593 (4)	49 (38)
C_2^{β}	4971 (7)	4181 (7)	2076 (3)	56 (4)
$C_2^{\gamma 1}$	5777 (9)	5583 (8)	2294 (5)	94 (6)
$C_2^{\gamma 2}$	5806 (8)	3681 (9)	1844 (4)	79 (6)
C_2'	3189 (10)	4639 (8)	1838 (4)	86 (5)
O_2'	2284 (7)	4111 (7)	2184 (4)	117 (5)
O_2	3605 (6)	5824 (5)	1622 (3)	111 (4)
C_3	2980 (14)	6471 (13)	1859 (6)	208 (11)

$$U_{eq^*} = 1/3\left[U_{11}(a\,a^*)^2 + U_{22}(b\,b^*)^2 + U_{33}(c\,c^*)^2 + 2U_{12}\,a\,b\,a^*\,b^*\cos\gamma + 2U_{23}\,b\,c\,c^*\,b^*\cos\alpha + 2U_{13}\,a\,c\,a^*\,c^*\cos\beta\right]$$

[b]The z coordinate of O_1' was kept fixed during refinement

Table 2D. Final atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for non-hydrogen atoms in methanol ($\varepsilon_r = 32.00$).

Atom	x	y	z	U_{eq^*}
C_1	10500 (10)	13894 (10)	730 (6)	60 (6)
C_2	9735 (9)	12507 (9)	907 (4)	51 (5)
O_1	8974 (7)	12098 (6)	1346 (3)	73 (4)
N_1	9936 (6)	11690 (7)	566 (4)	49 (4)
C_1^{α}	9109 (8)	10317 (8)	632 (4)	39 (4)
C_1^{β}	9491 (9)	9443 (9)	753 (4)	64 (5)
C_1^{γ}	10800 (9)	9661 (9)	852 (4)	57 (5)
$C_1^{\delta 1}$	10860 (12)	8524 (11)	976 (6)	89 (7)
$C_1^{\varepsilon 1}$	12069 (16)	8649 (15)	1160 (8)	139 (12)
C_1^{ζ}	13180 (12)	9805 (14)	1133 (7)	10 (9)
$C_1^{\varepsilon 2}$	13097 (11)	10833 (15)	1031 (7)	79 (9)

Table 2D *(Cont'd.)*

$C_1^{\delta 2}$	11960 (11)	10825 (11)	875 (6)	84 (8)
C_1'	7691 (8)	9832 (8)	555 (4)	44 (4)
$O_1'^{b}$	6855 (5)	8891 (6)	860	44 (4)
N_2	7328 (7)	10405 (7)	143 (4)	51 (4)
C_2^{α}	5978 (9)	9892 (9)	22 (4)	58 (5)
C_2^{β}	5818 (10)	10775 (10)	473 (5)	72 (6)
$C_2^{\gamma 1}$	4423 (12)	10181 (14)	676 (6)	118 (9)
$C_2^{\gamma 2}$	6325 (11)	12106 (11)	206 (5)	90 (7)
C_2'	5387 (10)	8511 (11)	228 (6)	74 (7)
O_2'	5866 (9)	8140 (9)	-570 (5)	134 (6)
C_3	3593 (18)	6462 (14)	-241 (8)	135 (13)

$U_{eq}^{a} = 1/3\left[U_{11}(a\,a^*)^2 + U_{22}(b\,b^*)^2 + U_{33}(c\,c^*)^2 + 2U_{12}\,a\,b\,a^*\,b^*\cos\gamma\right.$

$\left. 2U_{23}\,b\,c\,b^*\,c^*\cos\alpha + 2U_{13}\,a\,c\,a^*\,c^*\cos\beta\right]$

[b]The z coordinate of O_1' was kept fixed during refinement.

Table 2E. Final atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for non-hydrogen atoms in Acetonitrile ($\varepsilon_r = 36.02$).

Atom	x	y	z	U_{eq}^{a}
C_1	3868 (9)	3406 (10)	356 (10)	94 (7)
C_2	2495 (6)	2772 (7)	-517 (10)	72 (4)
O_1	2095 (5)	3153 (5)	-953 (9)	82 (3)
N_1	1668 (5)	1754 (5)	-166 (9)	64 (3)
C_1^{α}	338 (7)	1232 (7)	-256 (9)	69 (4)
C_1^{β}	-535 (7)	-31 (7)	364 (9)	88 (4)
C_1^{γ}	-358 (7)	-1172 (7)	-475 (9)	71 (4)
$C_1^{\delta 1}$	-1472 (9)	-2346 (8)	-595 (10)	99 (6)
$C_1^{\varepsilon 1}$	-1440 (11)	3442 (9)	-745 (10)	110 (7)
C_1^{ζ}	-180 (13)	-3342 (10)	-737 (10)	145 (7)
$C_1^{\varepsilon 2}$	852 (11)	-2232 (11)	-617 (11)	152 (8)
$C_1^{\delta 2}$	821 (9)	-1143 (9)	-492 (10)	137 (6)
C_1'	-204 (7)	2091 (7)	-162 (9)	78 (4)
$O_1'^{b}$	-1127 (5)	2040 (5)	-486	113 (3)
N_2	377 (6)	3058 (5)	258 (9)	96 (3)
C_2^{α}	89 (7)	3929 (7)	383 (9)	80 (4)

Table 2E (*Cont'd.*)

C_2^{β}	805 (8)	4981 (8)	844 (9)	89 (5)
$C_2^{\gamma 1}$	2144 (11)	5817 (9)	600 (10)	115 (7)
$C_1^{\gamma 2}$	244 (9)	5813 (9)	1048 (10)	131 (6)
C_2'	1475 (8)	3160 (9)	598 (9)	112 (6)
O_2'	1818 (7)	2248 (7)	976 (9)	120 (6)
O_2	2212 (6)	3605 (6)	378 (9)	131 (4)

$$U_{eq} = 1/3 \left[U_{11}(a\,a^*)^2 + U_{22}(b\,b^*)^2 + U_{33}(c\,c^*)^2 + 2U_{12}\,a\,b\,a^* b^* \cos\gamma + 2U_{23}\,b\,c\,c^*\,b^* \cos\alpha + 2U_{13}\,a\,c\,a^*\,c^* \cos\beta \right]$$

[b]The z coordinate of O_1' was kept fixed during refinement.

Table 2F. Final atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for non-hydrogen atoms in DMSO ($\varepsilon_r = 46.60$).

Atom	x	y	z	B
C_1	3628 (49)	−288 (49)	911 (22)	68
C_2	2688 (48)	125 (49)	746 (25)	69
O_1	3198 (31)	1115 (35)	308 (17)	84
N_1	1751 (34)	122 (38)	1092 (18)	53
C_1^{α}	1341 (58)	908 (60)	1011 (26)	80
C_1^{β}	120 (41)	627 (39)	893 (20)	28
C_1^{γ}	−1089 (40)	−841 (37)	787 (19)	24
$C_1^{\delta 1}$	−1014 (45)	−1907 (41)	741 (21)	47
$C_1^{\varepsilon 1}$	−2231 (28)	−3037 (27)	612 (17)	51
C_1^{ζ}	−3563 (54)	−3355 (59)	527 (28)	87
$C_1^{\varepsilon 2}$	−3358 (74)	−2136 (82)	529 (35)	154
$C_1^{\delta 2}$	−2388 (55)	−833 (50)	621 (25)	68
C_1'	2090 (47)	2210 (45)	1022 (22)	43
$O_1'^{b}$	2110 (27)	3311 (27)	75	48
N_2	3098 (27)	2639 (27)	1468 (16)	21
C_2^{α}	3884 (54)	4029 (54)	1647 (25)	72
C_2^{β}	5012 (46)	4178 (46)	2085 (22)	56
$C_2^{\gamma 1}$	5761 (42)	5647 (43)	2327 (21)	51
$C_2^{\gamma 2}$	5684 (64)	3663 (67)	1840 (31)	126

Table 2F *(Cont'd.)*

C_2'	3305 (50)	4664 (49)	1895 (24)	65
O_2	2208 (45)	5668 (41)	1654 (20)	102
C_3	2878 (60)	6556 (59)	1937 (29)	120

[b]The z coordinate of O_1' was kept fixed during refinement.

3. Results and discussion

The bond lengths and angles (Table 3) show that the geometry of the peptide remains unchanged in different solvents. The values in DMSO, due to poor quality of the data, show

Table 3. Bond lengths (Å) and valence angles (°) in (A) C_6H_6 (B) $CHCl_3$ (C) $CH_3COOC_2H_5$ (D) CH_3OH and (E) CH_3CN (F) $(CH_3)_2SO$ for non-hydrogen atoms.

	(A)	(B)	(C)	(D)	(E)	(F)
$C_1 - C_2$	1.49 (1)	1.47 (2)	1 50 (2)	1.48 (1)	1 46 (1)	1 47 (9)
$C_2 - O_1$	1.23 (1)	1.22 (1)	1 25 (1)	1.24 (1)	1.25 (2)	1.40 (6)
$C_2 - N_1$	1.35 (2)	1.31 (1)	1 33 (1)	1.34 (2)	1 35 (1)	1.35 (7)
$N_1 - C_1^{\alpha}$	1.41 (1)	1.45 (1)	1.42 (2)	1.43 (1)	1.39 (1)	1.27 (9)
$C_1^{\alpha} - C_1^{\beta}$	1 30 (1)	1 42 (1)	1.34 (1)	1.35 (2)	1.35 (1)	1 34 (9)
$C_1^{\beta} - C_1^{\gamma}$	1.51 (1)	1.40 (1)	1.42 (1)	1 46 (2)	1.49 (2)	1 63 (5)
$C_1^{\gamma} - C_1^{\delta 1}$	1.39 (1)	1 36 (2)	1.37 (1)	1 38 (1)	1.39 (1)	1.32 (8)
$C_1^{\delta 1} - C_1^{\varepsilon 1}$	1.30 (2)	1 35 (2)	1.39 (1)	1.39 (2)	1 36 (2)	1.42 (4)
$C_1^{\varepsilon 1} - C_1^{\zeta}$	1.43 (2)	1.33 (2)	1 35 (2)	1.29 (3)	1.44 (2)	1.44 (7)
$C_1^{\zeta} - C_1^{\varepsilon 2}$	1.30 (2)	1.33 (2)	1.34 (2)	1 35 (2)	1.30 (2)	1.34 (12)
$C_1^{\varepsilon 2} - C_1^{\delta 2}$	1.42 (2)	1.44 (2)	1.37 (1)	1.43 (3)	1.34 (2)	1.41 (9)
$C_1^{\delta 2} - C_1^{\gamma}$	1.34 (1)	1.42 (2)	1.41 (1)	1 42 (2)	1.38 (2)	1 59 (9)
$C_1^{\alpha} - C_1'$	1.46 (1)	1 50 (2)	1.52 (1)	1.49 (1)	1.47 (2)	1 35 (7)
$C_1' - O_1'$	1.28 (1)	1.22 (1)	1.20 (1)	1.25 (1)	1.28 (2)	1.42 (7)
$C_1' - N_2$	1.37 (1)	1.34 (1)	1.35 (1)	1.33 (1)	1 36 (2)	1.43 (6)
$N_2 - C_2^{\alpha}$	1.45 (1)	1.43 (1)	1.44 (1)	1.43 (1)	1.42 (1)	1.49 (6)
$C_2^{\alpha} - C_2^{\beta}$	1.52 (1)	1.52 (2)	1.55 (1)	1.59 (2)	1.54 (2)	1.58 (8)
$C_2^{\beta} - C_2^{\gamma 1}$	1.51 (2)	1.50 (2)	1.52 (1)	1.50 (2)	1.49 (2)	1.34 (11)
$C_2^{\beta} - C_2^{\gamma 2}$	1.50 (1)	1.57 (2)	1.48 (2)	1.51 (2)	1.51 (2)	1.60 (7)
$C_2^{\alpha} - C_2'$	1.54 (1)	1.53 (2)	1.51 (2)	1.53 (2)	1.50 (1)	1.36 (10)
$C_2' - O_2'$	1.23 (1)	1.20 (2)	1.20 (1)	1.15 (2)	1.26 (2)	1.34 (7)
$C_2' - O_2$	1.36 (1)	1.31 (1)	1.32 (1)	1.34 (1)	1.32 (2)	1.26 (9)
$O_2 - C_3$	–	1.42 (2)	1.41 (2)	1.45 (2)	–	1.59 (10)

Table 3 (*Cont'd*). Bond angles (°) in the peptides for non-hydrogen atoms.

	(A)	(B)	(C)	(D)	(E)	(F)
$C_1 - C_2 - O_1$	122 (1)	123 (1)	121 (1)	124 (1)	122 (1)	111 (5)
$O_1 - C_2 - N_1$	122 (1)	120 (1)	124 (1)	121 (1)	121 (1)	113 (5)
$C_1 - C_2 - N_1$	116 (1)	117 (1)	115 (1)	115 (1)	117 (1)	129 (5)
$C_2 - N_1 - C_1^{\alpha}$	118 (7)	120 (1)	117 (1)	120 (1)	118 (1)	122 (5)
$N_1 - C_1^{\alpha} - C_1^{\beta}$	125 (1)	125 (1)	117 (1)	126 (1)	126 (1)	128 (5)
$N_1 - C_1^{\alpha} - C_1'$	115 (1)	117 (1)	116 (1)	115 (1)	118 (1)	124 (6)
$C_1' - C_1^{\alpha} - C_1^{\beta}$	119 (1)	118 (1)	119 (1)	118 (1)	116 (1)	108 (5)
$C_1^{\alpha} - C_1^{\beta} - C_1^{\gamma}$	134 (1)	129 (1)	132 (1)	129 (1)	131 (1)	124 (5)
$C_1^{\beta} - C_1^{\gamma} - C_1^{\delta 1}$	117 (1)	130 (1)	126 (1)	129 (1)	117 (1)	126 (4)
$C_1^{\beta} - C_1^{\gamma} - C_1^{\delta 2}$	123 (1)	113 (1)	117 (1)	115 (1)	126 (1)	111 (3)
$C_1^{\delta 1} - C_1^{\gamma} - C_1^{\delta 2}$	120 (1)	116 (1)	116 (1)	117 (1)	118 (1)	121 (4)
$C_1^{\gamma} - C_1^{\delta 1} - C_1^{\epsilon 1}$	122 (1)	124 (1)	121 (1)	120 (1)	123 (1)	113 (4)
$C_1^{\delta 1} - C_1^{\epsilon 1} - C_1^{\zeta}$	116 (1)	121 (2)	120 (1)	125 (2)	116 (1)	137 (4)
$C_1^{\epsilon 1} - C_1^{\zeta} - C_1^{\epsilon 2}$	123 (1)	119 (1)	121 (1)	118 (2)	120 (1)	98 (6)
$C_1^{\zeta} - C_1^{\epsilon 2} - C_1^{\delta 2}$	118 (1)	120 (1)	120 (1)	121 (2)	124 (1)	142 (9)
$C_1^{\epsilon 2} - C_1^{\delta 2} - C_1^{\gamma}$	119 (1)	120 (2)	121 (1)	119 (1)	120 (1)	107 (6)
$C_1^{\alpha} - C_1' - O_1'$	123 (1)	122 (1)	124 (1)	121 (1)	124 (1)	138 (5)
$O_1' - C_1' - N_2$	119 (1)	124 (1)	121 (1)	120 (1)	118 (1)	109 (3)
$C_1^{\alpha} - C_1' - N_2$	118 (1)	115 (1)	115 (1)	118 (1)	118 (1)	113 (4)
$C_1' - N_2 - C_2^{\alpha}$	121 (1)	119 (1)	119 (1)	119 (1)	122 (1)	122 (4)
$N_2 - C_2^{\alpha} - C_2^{\beta}$	110 (1)	111 (1)	110 (1)	109 (1)	111 (1)	108 (4)
$C_2^{\beta} - C_2^{\alpha} - C_2'$	105 (1)	112 (1)	109 (1)	109 (1)	113 (1)	109 (5)
$N_2 - C_2^{\alpha} - C_2'$	105 (1)	109 (1)	108 (1)	110 (1)	109 (1)	121 (5)
$O_2' - C_2' - O_2$	126 (1)	126 (2)	123 (1)	124 (1)	126 (1)	109 (5)
$C_2^{\alpha} - C_2^{\beta} - C_2^{\gamma 1}$	109 (1)	110 (1)	111 (1)	108 (1)	112 (1)	112 (5)
$C_2^{\alpha} - C_2' - O_2'$	124 (1)	123 (2)	125 (1)	126 (1)	121 (1)	122 (5)
$C_2^{\alpha} - C_2' - O_2$	109 (1)	111 (1)	113 (1)	110 (1)	113 (1)	122 (5)
$C_2^{\alpha} - C_2^{\beta} - C_2^{\gamma 2}$	112 (1)	110 (1)	111 (1)	111 (1)	112 (2)	107 (4)
$C_2^{\gamma 1} - C_2^{\beta} - C_2^{\gamma 2}$	112 (1)	113 (1)	111 (1)	112 (1)	112 (1)	120 (5)
$C_2' - O_2 - C_3$	–	112 (2)	115 (1)	111 (1)	–	126 (5)

Table 4. Torsional angles (°) in (A) C_6H_6 (B) $CHCl_3$ (C) $CH_3COOC_2H_5$ (D) CH_3OH (E) CH_3CN and (F) $(CH_3)_2SO$.

		(A)	(B)	(C)	(D)	(E)	(F)
ω_0	$C_1 - C_2 - N_1 - C_1^{\alpha}$	173 (1)	−169 (1)	174 (1)	−170 (1)	172 (1)	153 (6)
ϕ_1	$C_2 - N_1 - C_1^{\alpha} - C_{1'}$	−60 (2)	54 (2)	−56 (1)	55 (1)	−60 (2)	−59 (9)
ψ_1	$N_1 - C_1^{\alpha} - C_1 - N_2$	−34 (2)	37 (2)	−36 (1)	36 (1)	−31 (2)	−34 (8)
ω_1	$C_1^{\alpha} - C_1' - N_2 - C_2^{\alpha}$	−180 (1)	175 (1)	−177 (1)	175 (1)	−179 (1)	−169 (5)
ϕ_2	$C_1' - N_2 - C_2^{\alpha} - C_2'$	67 (2)	−60 (2)	65 (1)	−62 (1)	59 (2)	60 (7)
θ_2^T	$N_2 - C_2^{\alpha} - C_2' - O_2$	−141 (1)	140 (1)	−141 (1)	143 (1)	−141 (1)	−122 (6)
χ_1	$N_1 - C_1^{\alpha} - C_1^{\beta} - C_1^{\gamma}$	−5 (3)	−1 (2)	−7 (1)	1 (2)	−6 (3)	−7 (9)
$\chi_{1'}$	$C_{1'} - C_1^{\alpha} - C_1^{\beta} - C_1^{\gamma}$	−177 (2)	−178 (1)	176 (1)	−179 (1)	−180 (1)	176 (4)
$\chi_1^{2,2}$	$C_1^{\alpha} - C_1^{\beta} - C_1^{\gamma} - C_1^{\delta 1}$	−5 (3)	2 (3)	3 (1)	4 (2)	−1 (2)	−6 (8)
$\chi_1^{2,1}$	$C_1^{\alpha} - C_1^{\beta} - C_1^{\gamma} - C_1^{\delta 2}$	−175 (2)	−178 (1)	−179 (1)	173 (1)	−178 (2)	−176 (5)
$\chi_2^{1,1}$	$N_2 - C_2^{\alpha} - C_2^{\beta} - C_2^{\gamma 1}$	67 (2)	−62 (2)	65 (1)	−64 (1)	63 (2)	52 (6)
$\chi_2^{1,2}$	$N_2 - C_2^{\alpha} - C_2^{\beta} - C_2^{\gamma 2}$	−168 (1)	172 (1)	170 (1)	−173 (1)	−173 (2)	−174 (4)
$\psi_{1'}$	$O_1' - C_1' - C_1^{\alpha} - N_1$	148 (2)	−143 (1)	148 (1)	−145 (1)	144 (2)	152 (6)
	$O_{1'} - C_{1'}\ C_1^{\alpha} - C_1^{\beta}$	−38 (2)	36 (1)	35 (1)	35 (1)	−42 (2)	−31 (9)
$\nu^{0,1}$	$O_{1'} - C_{1'} - N_2 - C_2^{\alpha}$	1 (2)	−6 (2)	1 (1)	−4 (1)	6 (2)	7 (6)
$\psi_{2'}$	$O_{2'} - C_{2'} - C_2^{\alpha} - N_2$	41 (3)	−45 (2)	38 (1)	−40 (2)	43 (2)	29 (9)
	$O_{2'} - C_{2'} - C_2^{\alpha} - C_2^{\beta}$	−77 (2)	78 (2)	−82 (1)	80 (2)	−80 (2)	−97 (6)
	$O_2 - C_{2'} - C_2^{\alpha} - N_2$	−141 (1)	140 (1)	141 (1)	143 (1)	−140 (1)	129 (6)
	$O_2 - C_{2'} - C_2^{\alpha} - C_2^{\beta}$	101 (2)	−96 (2)	−100 (1)	−98 (1)	96 (2)	−110 (7)

large experimental errors. The characteristic ORTEP drawing of the peptide is illustrated in Figure 1. The peptide adopts a structure with Φ, Ψ values of the order of ±50, ±50° in various solvents. As seen from Table 4, the torsion angles Φ, Ψ remain unaltered in all these

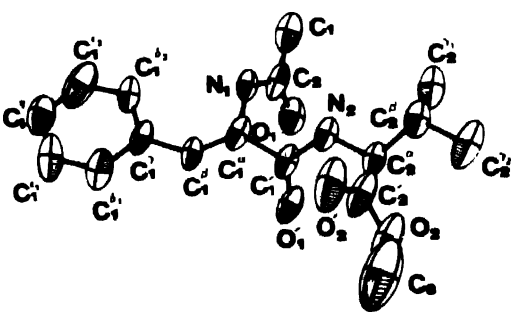

Figure 1. The ORTEP drawing of the molecule with thermal ellipsoids at 50% probability.

solvents. The only observable change is found in the torsion angles involving terminal oxygen atoms where the values in DMSO differ only slightly from those found in other solvents. This suggests that the conformation of the peptide does not seem to change appreciably in various solvents of widely different polarities. The molecular packing in the unit cell as seen along the *a*-axis is shown in Figure 2. The molecules are arranged in the form

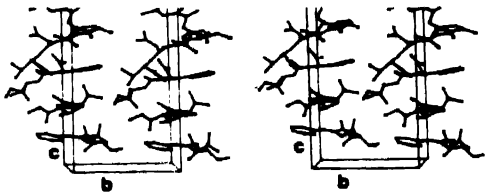

Figure 2. The stereoscopic view of the molecular packing of the peptide in the crystals.

of helices along the c-axis. The molecules are linked with each other by two hydrogen bonds which are parallel to the *c*-axis: $N_1 - H_1$ -----O_1 (y, y–x, 0.17 + z) and $N_2 - H_2$ ----- O_1' (y, y–x, 0.17 + z) with distances ranging between 2.80 to 3.10 Å. In the plane perpendicular to the *c*-axis, the molecules are held together by van der Waals interactions involving N_1, O_1; C_2, O_1; and C_1, O_2 with distances varying between 3.25 to 3.50 Å.

Spectral assignment :

The dehydro-Phe (NH) is identified easily as a singlet in the low field region because of the absence of C^{α} proton. Also, the dehydro-Phe (C^{β}) appears at a relatively low field due to a similar reason. The C^{α}, C^{β}, C^{γ} protons corresponding to the Val residue are identified from the double quantum filter spectra (not shown here).

NOE data :

All the intra residue NOEs between protons of Val residue are observed suggesting that the peptide is present as one major conformer. The only major inter residue NOE observed is of the type N_iH.....$N_{i+1}H$ between Phe (NH) and Val (NH). This NOE is indicative of folded conformation in peptides [32]. It is of almost equal intensity both in $CDCl_3$ (Figure 3) and in DMSO (Figure 4). Thus, it can be said that the conformation around dehydro-Phe residue does not change appreciably in solvents of different polarities. However, because of short length of the peptide more NOE cross peaks are not observed. The intensities of the NOE cross peaks match with the distances obtained from crystal structures of this peptide which suggest that the structure of the peptide in solution is similar to that observed in solid state. Thus, it suggests that the conformation of the peptide N-Ac-dehydro-Phe-L-Val-OCH_3 remains unchanged in various solvents both in solid as well as in solution states.

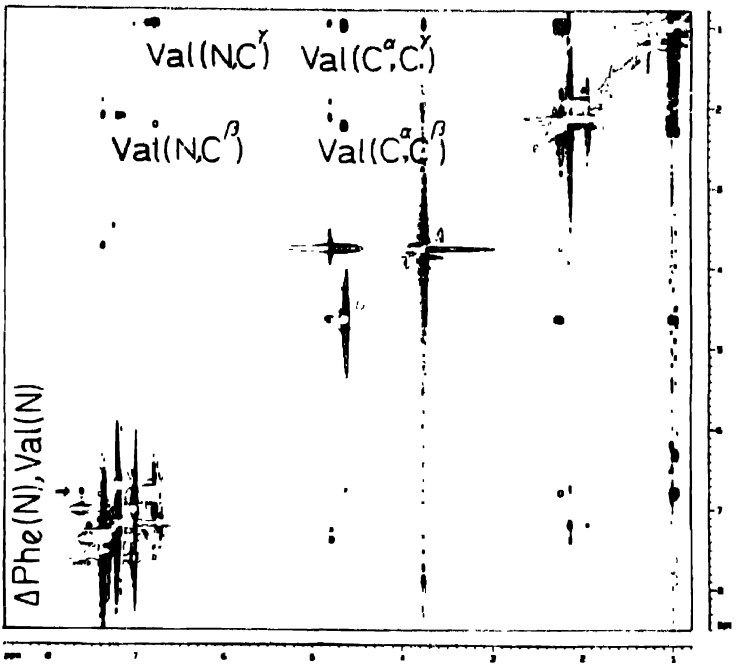

Figure 3. The ROESY spectrum of the peptide in $CDCl_3$.

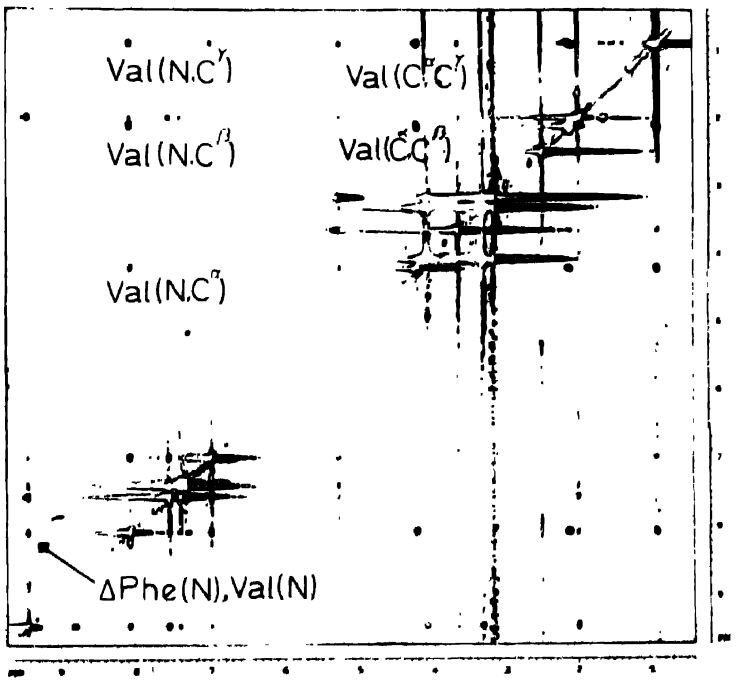

Figure 4. The ROESY spectrum of the peptide in DMSO.

4. Conclusions

It seems to be clear that the torsion angles at the dehydro-residue and to a large extent of its neighbouring residues are primarily governed by the steric constraints of the dehydro-residue. The solvent effects appear to be relatively smaller in comparison to the magnitudes of steric forces. It is expected that the most dominant conformation in solution phase is likely to be trapped in crystalline state. Since all the crystal forms from different solvents are similar, the conformation of the peptide in these solvents must be identical in solution. This situation must be similar to seeing a dominant conformation of peptide in different solvents. Thus the present studies demonstrate that the short dehydro-residue containing peptides adopt identical conformations under widely different solvent conditions. Therefore, a dehydro-residue introduces strong steric effects, which play a crucial role in nucleating a specific conformation in a peptide. This property of a dehydro-residue can be exploited to generate very specific structures of peptides as observed from the studies carried out so far :

1. A dehydro-Phe/Leu/Abu at the (i + 2) position in a three- peptide unit sequence induces β-turn II conformation with an $i + 3 \rightarrow i$ hydrogen bond [1,6-8,15,16,17].
2. A dehydro-residue at the (i + 1) position with a flexible residue such as Gly or Ala at (i + 2) position produces the β-turn II conformation [1,10,16].
3. A dehydro-residue at (i + 1) position, with a bulky residue such as Leu, Val *etc.* at the (i + 2) position, is accommodated in an alternating left handed-right handed zigzag conformation [1,3,4].
4. If the dehydro-residues appear consecutively in a peptide sequence, the resulting conformation is a planar zigzag structure [1,16].
5. A sequence containing two or more dehydro-residues, which are separated by one or two saturated residues, result in a three 3_{10}-helical conformation [1,2,5,9,14,16].
6. The dehydro-Ala has only a $-CH_2$ as its side chain. The steric effects caused by this residue are small, which can be released through an allowed distortion in geometry. The N–C^{α}–C' angle in dehydro-Ala is of the order of 110°, which is significantly smaller as compared to an average value of 117° in other dehydro-residues and 120° in a planar structure. With the neutralization of steric constraints through distortion in geometry, a planar dehydro-Ala adopts a preferable extended conformation with Φ, Ψ values of the order of ± 180°, ± 180° to compensate for the loss of energy caused by the distortion in the geometry [1,12,13,16].
7. The constraints introduced by dehydro-Val are much more pronounced than those observed in dehydro-Phe, dehydro-Leu, dehydro-Abu and dehydro-Ala. This is because the side chain branching in dehydro-Val occurs at the C^{β} atom itself. As a result of this, dehydro-Val at the (i + 2) position, unlike dehydro-Phe, can not be

accommodated in a β-turn II conformation. Therefore, it produces a more folded β-turn III conformation in a sequence of Xxx–Yyy-dehydro-Val-Zzz [11,16].

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